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The frequency and association of intra-amniotic inflammation with perinatal outcome in twin pregnancies with preterm labor and intact membranes

조기진통을 동반한 쌍태임신에서
양수내 염증의 빈도 및
주산기 예후와의 관련성

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Abstract

The frequency and association of intra-amniotic inflammation with perinatal outcome in twin pregnancies with preterm labor and intact membranes

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Objectives

Twin pregnancies have a six-fold higher perinatal mortality compared with singleton pregnancies. Preterm delivery is the major cause of perinatal mortality and morbidity in twin pregnancies. The frequency of intra-amniotic inflammation (IAI) reaches 30% of singleton pregnancies with preterm labor and intact membranes. Moreover, IAI alone (regardless of the presence or absence of proven intra-amniotic infection) is a risk factor for the progression of labor to preterm delivery and adverse outcome. The purpose of this study was to evaluate the

frequency and association of IAI with perinatal outcome in twin pregnancies with preterm labor.

Methods

Amniotic fluid (AF) was retrieved from both sacs in 90 twin gestations with preterm labor and intact membranes (gestational age between 20^{+0} and 34^{+6} weeks). Fluid was cultured for aerobic and anaerobic bacteria and genital mycoplasmas and assayed for matrix metalloproteinase-8 (MMP-8). Microbial invasion of amniotic cavity (MIAC) was diagnosed in cases with a positive AF culture. IAI was defined as an AF MMP-8 concentration >23 ng/mL. Study population was divided into 3 groups according to the results of AF analysis: group 1, cases without IAI or MIAC (n=55); group 2, cases without MIAC but with IAI of at least 1 amniotic cavity (n=26); group 3, cases with IAI and MIAC of at least 1 amniotic cavity (n=9). Non-parametric and survival techniques were used for analysis. Multiple logistic analysis and generalized estimation equation models were used to adjust important confounding variables.

Results

1) The rate of IAI of at least 1 amniotic cavity was 39% (35/90), among which IAI with MIAC was detected in 10% (9/90), and IAI without MIAC was found in 29% (26/90); 2) IAI was present in both sacs in 22 cases, in the presenting sac in 12 cases, and in the non-presenting sac in 1 case; 3) AF culture was positive in both sacs in 6 cases and in the presenting sac in 3 cases; 4) Women without MIAC but with IAI of at least 1 amniotic cavity (group 2) had a significantly higher rate of adverse outcome (a lower gestational age at delivery, shorter amniocentesis-to-delivery interval, and neonatal death and/or any significant morbidity) than those

without IAI or MIAC (group 1). There was no significant difference in pregnancy outcomes between women without MIAC but with IAI of at least 1 amniotic cavity (group 2) and those with IAI and MIAC of at least 1 amniotic cavity (group 3).

Conclusion

1) IAI is present in 39% of twin pregnancies with preterm labor and intact membranes and is a risk factor for impending preterm delivery and adverse outcome. 2) IAI was found more frequently in the presenting sac than in the non-presenting sac, which supports the view that the ascending route is the common pathway of intra-amniotic infection/inflammation.

Key words: intra-amniotic inflammation; intra-amniotic infection; preterm labor; preterm birth; twin pregnancy

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Contents

List of Tables

List of Figure

Introduction

Twin pregnancies have a six-fold higher perinatal mortality compared with singleton pregnancies.[1] Preterm delivery is the major cause of perinatal mortality and morbidity in twin pregnancies.[2-5] Greater than one-half of twins are born prior to 37 completed weeks of gestation,[2,5-7] and the rate of very preterm births (< 32 weeks' gestation) is 12% in twin pregnancies, which is 7 times higher than that in singleton pregnancies.[8] Spontaneous preterm labor is one of the major causes of preterm births in twins, as well as singletons.[1,2]

Substantial evidence suggests that intra-amniotic infection in women with preterm labor and intact membranes is a risk factor for the progression of labor to preterm delivery despite tocolysis, impending preterm delivery and adverse neonatal outcome.[9-15] Intra-amniotic infection is present in approximately 10% of singleton pregnancies with preterm labor and intact membranes.[11-13, 16-20] The frequency of intra-amniotic infection is approximately 10% in twin pregnancies presenting with preterm labor and intact membranes,[21-22] which is comparable to that in singleton pregnancies. The gold standard for the diagnosis of intra-amniotic infection in clinical medicine is the isolation of the microorganisms from the amniotic fluid (AF). However, a strong body of evidence suggests that a negative culture cannot be considered to definitively exclude intra-amniotic infection.[23-26] Moreover, the results of microbial culture may take several days, and are often not available in time for clinical decisions.

In contrast to the diagnosis of infection using culture techniques, the identification of intra-amniotic inflammation can be easily and rapidly detected by determinations of white blood cell count,[18,27] proinflammatory cytokines[28-39] or matrix metalloproteinases levels.[40-48] Our previous study demonstrated that the

frequency of intra-amniotic inflammation reaches 30% of singleton pregnancies with preterm labor and intact membranes and 50% of those with subsequent preterm birth.[12] Moreover, intra-amniotic inflammation alone (regardless of the presence or absence of proven intra-amniotic infection) is a risk factor for the progression of labor to preterm delivery and adverse outcome in singleton pregnancies.[12,13,31,40,49-55] However, there is a paucity of studies regarding the frequency and clinical implications of intra-amniotic inflammation in twin pregnancies with preterm labor and intact membranes. The purpose of this study was to evaluate the frequency and association of intra-amniotic inflammation with perinatal outcome in twin pregnancies with preterm labor.

Materials and Methods

Study Population

The study population consisted of consecutive women with twin pregnancies who were admitted to the Seoul National University Hospital with a diagnosis of preterm labor and intact membranes and who underwent amniocentesis of both amniotic cavities for assessment of the microbiologic status of the amniotic cavity and/or fetal lung maturity between January 1993 and December 2012. Preterm labor was defined as the presence of painful and regular uterine contractions with a frequency of at least 2 every 10 minutes requiring hospitalization. The inclusion criteria were: (1) twin gestation with live fetuses; (2) preterm labor with intact membranes; (3) gestational age at amniocentesis between 20^{+0} and 34^{+6} weeks of gestation. Rupture of membranes was excluded by testing pooling and nitrazine paper reaction. According to hospital protocol, amniocentesis is offered routinely to all women who are admitted with a diagnosis of preterm labor. AF was retrieved after written informed consent was obtained. The results of AF were not blinded to the physicians. The administration of antibiotics and tocolytics was left to the physician's discretion. The Institutional Review Board of Seoul National University Hospital approved the collection and use of these samples and information for research purposes. The Seoul National University Hospital has received a Federal Wide Assurance with the Office for Human Research Protection of the Department of Health and Human Services of the United States.

AF study

AF was retrieved by transabdominal amniocentesis from both sacs and was cultured for aerobic and anaerobic bacteria and genital mycoplasmas (ureaplasmas and *Mycoplasma hominis*). Microbial invasion of amniotic cavity (MIAC) was defined as a positive AF culture. The remaining fluid was centrifuged and stored in polypropylene tubes at -70°C. The stored AF was analyzed for matrix metalloproteinase (MMP)-8, which was measured with a commercially available enzyme-linked immunosorbent assay (R and D Systems, Inc., Minneapolis, MN, USA). Each measurement was done in duplicate. Intra- and inter-assay coefficients were <10% each. Intra-amniotic inflammation was defined as an elevated AF MMP-8 concentration (>23 ng/mL), as previously reported.[46]

Women were divided into 3 groups according to the presence or absence of intra-amniotic inflammation and/or MIAC: 1) Group 1 consisted of 55 women without intra-amniotic inflammation or MIAC; 2) group 2 consisted of 26 women without MIAC but with intra-amniotic inflammation of at least 1 amniotic cavity; and 3) group 3 consisted of 9 women with intra-amniotic inflammation and MIAC of at least 1 amniotic cavity.

Diagnosis of acute histologic chorioamnionitis, funisitis, clinical chorioamnionitis and neonatal morbidity

Acute histologic chorioamnionitis was defined in the presence of acute inflammatory changes in the chorion-decidua and/or amnion; funisitis was diagnosed in the presence of neutrophil infiltration into umbilical vessel walls or Wharton's jelly with use of criteria published previously.[30] Clinical chorioamnionitis was diagnosed when a temperature was elevated to 37.8℃ and

when ≥ 2 of the following criteria were present: uterine tenderness, malodorous vaginal discharge, maternal leukocytosis (>15,000 cells/mm^3), maternal tachycardia (>100 beats/min), and fetal tachycardia (>160 beats/min) as proposed by Gibbs et al.[56]

Significant neonatal morbidity was defined as the presence of any of the following conditions: proven early neonatal sepsis, respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage, and necrotizing enterocolitis.[54] The diagnosis of respiratory distress syndrome was defined as the presence of respiratory distress, increased oxygen requirement (FiO_2 > 0.4), and diagnostic radiological and laboratory findings of reticulogranular patterns in the absence of evidence of any other causes of respiratory distress. Bronchopulmonary dysplasia was defined as the treatment with oxygen >21 percent for at least 28 days of age using the criteria of the National Institute of Child Health Workshop definition.[57] Proven early neonatal sepsis was diagnosed as the isolation of a bacterium from a blood culture in the first 72 hours of age. Intraventricular hemorrhage was defined as Grade II or higher by McMenamin's classification of periventricular-intraventricular hemorrhage.[58] The diagnosis of necrotizing enterocolitis was based on the presence of the characteristic clinical features of abdominal distention and the abdominal radiographic finding of pneumatosis intestinalis.

Statistical analysis

Proportions were compared with the use of the Fisher's exact test. The Kruskal-Wallis analysis of variance test was used for comparison of continuous variables among groups. Multiple comparisons among groups were performed with

the Mann-Whitney U test and adjusted using the Bonferroni method. The McNemar test and the Wilcoxon signed rank test were used for comparison for the analysis of the matched data in a twin pair. The amniocentesis-to-delivery interval was compared with the use of the generalized Wilcoxon test for survival analysis. A Cox proportional hazard model was used to control covariates. The amniocentesis-to-delivery interval of women who were delivered for maternal or fetal indications was treated as a censored observation, with the censoring time equal to the amniocentesis-to-delivery interval. Logistic regression analysis was used to examine the relationship between the presence of intra-amniotic inflammation and the pregnancy outcome of interest after adjusting for the effects of confounding variables including gestational age at amniocentesis and cervical dilatation which were associated with the occurrence of spontaneous preterm birth <34 weeks and adverse neonatal outcome (P<.01). Generalized estimation equation models were used to explore the association between the presence of intra-amniotic inflammation and neonatal outcomes adjusting for the effects of confounding variables while accounting for correlation due to clustering of multiple births within a mother. A probability value < 0.05 was considered as significant. In comparison among three groups, a probability value < 0.017 was considered as significant. SPSS 22.0 for Windows (IBM, Armonk, NY, USA) was used for statistical analyses.

Results

Characteristics of study population

A total of 98 women were met the inclusion criteria. Among them, AF was not available in 8 women for MMP-8 determinations (1 woman with a positive AF culture and 7 women with a negative culture); therefore, these women were excluded from further analysis because they could not be evaluated with respect to the presence or absence of intra-amniotic inflammation. Table 1 displays the clinical characteristics of the study population. Women without MIAC but with intra-amniotic inflammation of at least 1 amniotic cavity (group 2) had significantly more advanced cervical dilation than women without intra-amniotic inflammation or MIAC (group 1). There were no significant differences between women in groups 2 and 3.

AF culture

The frequency of MIAC of at least 1 amniotic cavity was 10% (9/90). Among 9 women with MIAC, microorganisms were isolated from both sacs in 6 cases and from the presenting sac alone in 3 cases. Ureaplasmas were the most common microorganisms isolated from the AF (from both sacs of 6 women). Other microorganisms recovered from the AF included *Mycoplasma hominis* (co-infection with Ureasplasmas from both sacs of 2 women), Group B *Streptococcus* (from the presenting sac of 1 patient), *Streptococcus viridans* (from the presenting sac of 1 patient), and *Staphylococcus capitis* (from the presenting sac of 1 patient).

Intra-amniotic inflammation

The frequency of intra-amniotic inflammation (defined as a MMP-8

concentration >23 ng/mL) of at least 1 amniotic cavity was 39% (35/90) of the study population. Intra-amniotic inflammation was present in all 9 women (100%) with MIAC, and in 26 of 81 women (32%) without MIAC. The presenting sac had a higher rate of intra-amniotic inflammation than the non-presenting sac (the presenting sac: 38% [34/90] vs. the non-presenting sac: 26% [23/90]; P = 0.003 by the McNemar test). Intra-amniotic inflammation was present in both sacs in 22 cases, in the presenting sac alone in 12 cases, and in the non-presenting sac alone in 1 case. The median AF MMP-8 concentration of the presenting sac was significantly higher than the non-presenting sac (median, 3.0 ng/mL [interquartile range, 0.6-86.6 ng/mL] vs. median, 1.6 ng/mL [interquartile range, 0.4-23.6 ng/mL]; P < 0.001 by the Wilcoxon signed rank test). Among the 35 women with intra-amniotic inflammation of at least 1 amniotic cavity, the presenting sac had a significantly higher median AF MMP-8 level than the non-presenting sac (median, 172.2 ng/mL [interquartile range, 65.1-353.0 ng/mL] vs. median, 57.0 ng/mL [interquartile range, 13.9-237.4 ng/mL]; P = 0.008 by the Wilcoxon signed rank test).

Pregnancy outcomes

Figure 1 shows the amniocentesis-to-delivery interval. In 11 women who were delivered because of maternal or fetal indications, this interval was censored. Women in group 2 had a significantly shorter median amniocentesis-to-delivery interval than did women in group 1 (P < 0.001); however, there was no difference in the median amniocentesis-to-delivery interval between groups 2 and 3. Table 2 shows the result of multivariate survival analysis. Women in group 2 had a significantly shorter median amniocentesis-to-delivery interval than women in

group 1 after adjusting for the gestational age at amniocentesis and the cervical dilation (hazard ratio, 4.14; 95% CI, 2.36-7.26; P < 0.001 by Cox proportional hazards model analysis).

Table 3 compares the pregnancy outcomes of the study population. Women in group 2 had significantly higher rates of spontaneous delivery within 1, 2 and 7 days of amniocentesis than did women in group 1 even after adjusting for the gestational age at amniocentesis and the cervical dilation (adjusted odds ratio [aOR], 6.81; 95% confidence interval [CI], 2.05-22.6; P = 0.002 for spontaneous delivery within 1 day: aOR, 11.2; 95% CI, 3.40-36.6; P < 0.001 for spontaneous delivery within 2 days: aOR, 19.8; 95% CI, 4.29-91.3; P < 0.001 for spontaneous delivery within 7 days by multiple logistic regression analysis). The rates of acute histologic chorioamnionitis and funisitis were higher in group 2 than in group 1 but the differences were statistically insignificant (P = 0.052 and 0.068, respectively).

Neonatal outcome

Among the study population, 6 cases with major congenital anomaly (n=4) or unavailable for neonatal data (n=2) were excluded from the analysis of neonatal outcome. Table 4 compares the neonatal outcome of the study population. Neonates born to mothers who had intra-amniotic inflammation of at least 1 amniotic cavity without MIAC (group 2) had a significantly higher rate of adverse neonatal outcome (neonatal death and/or any significant morbidity) than did those born to mothers without intra-amniotic inflammation or MIAC (group 1) even after adjusting for gestational age at amniocentesis and the cervical dilatation (P = 0.007). Table 5 showed the relationship of various variables with neonatal death and/or any significant morbidity. The presence of intra-amniotic inflammation

without MIAC, intra-amniotic inflammation with MIAC and gestational age at amniocentesis was significantly associated with the occurrence of adverse neonatal outcome. However, when adjusted for gestational age at delivery, all of these variables (the presence or absence of intra-amniotic inflammation and/or MIAC, cervical dilation, and gestational age at amniocentesis) were not associated with the occurrence of adverse neonatal outcome.

Table 6 described the pregnancy and neonatal outcomes according to the number of amniotic cavity with an elevated MMP-8 concentration. Cases with intra-amniotic inflammation of 1 amniotic cavity had a significantly higher rate of spontaneous delivery within 7 days than did those without intra-amniotic inflammation (85% [11/13] vs. 32% [17/53], P = 0.004 after adjusting for the gestational age at amniocentesis and cervical dilation). Cases with intra-amniotic inflammation of 1 amniotic cavity had a similar pregnancy and neonatal outcome to cases with intra-amniotic inflammation of both amniotic cavities except spontaneous delivery within 2 days.

Discussion

Principal findings of the study

(1) Intra-amniotic inflammation was present in 39% of twin pregnancies with preterm labor and intact membranes; (2) intra-amniotic inflammation per se is a risk factor for impending preterm delivery and adverse neonatal outcome regardless of AF culture results; and (3) intra-amniotic inflammation was more common in the presenting sacs than in the non-presenting sacs.

Intra-amniotic inflammation and twin pregnancy

Substantial evidence indicates that intra-amniotic inflammation plays an important role in the pathogenesis of preterm labor and subsequent preterm birth.[12,40,59-70] In the present study, intra-amniotic inflammation without MIAC was shown in 29% (26/90) of women with twin pregnancies who had preterm labor and intact membranes, and intra-amniotic inflammation without MIAC was more common than MIAC (10% [10/96]). These findings are in agreement with a previous report[12] made in singleton pregnancies with preterm labor and intact membranes. In singleton pregnancies with preterm labor and intact membranes, microbiologically-proven intra-amniotic infection occurred in 10% of women and intra-amniotic inflammation without proven AF infection was present in 21% of women.[12] Moreover, we found that intra-amniotic inflammation per se is a risk factor for the impending preterm birth and adverse neonatal outcome in twin pregnancies with preterm labor and intact membranes. The intra-amniotic inflammation in women without MIAC may result from a failure of detection of microorganisms by current microbiologic techniques,[23-26] an inflammatory

response caused by extra-amniotic infection [71,72] or non-infectious cause of inflammation.[73,74]

Our results showed that the frequency of intra-amniotic inflammation in twin pregnancies was similar to that of singleton pregnancies with preterm labor and intact membranes, but in contrast to the traditional view that the cause of a higher rate of preterm delivery in twin pregnancies is uterine overextension and myometrial stretching,[75,76] which can induce myometrial contractility[77] and oxytocin receptor in myometrium.[78] Recent studies have shown that the cervical length of women with twin pregnancies is shorter than the cervical length of women with singleton pregnancies,[79-82] and that short cervical length is associated with preterm birth[80, 83-92] and intra-amniotic inflammation/MIAC.[93-97] Collectively, these data suggest that the higher frequency of intra-amniotic inflammation in twin pregnancies may be due to the premature change in cervical length. Our data showed that intra-amniotic inflammation has an important role in the pathogenesis of preterm labor in twin pregnancies.

In the present study, intra-amniotic inflammation regardless of the presence or absence of MIAC occurred in 39% of twin pregnancies presenting with preterm labor and intact membranes. Moreover, 75% (21/28) of women who delivered within 48 hours of amniocentesis had intra-amniotic inflammation. However, 81% of women (40/49) without intra-amniotic inflammation/MIAC had a spontaneous preterm birth before 36 weeks of gestation, although they had a longer amniocentesis-to-delivery interval than women with intra-amniotic inflammation. It is partially at odds with the findings of a previous study,[12] in which only 35% (40/113) of singleton women with preterm labor and without intra-amniotic infection/inflammation had a spontaneous preterm birth (<37 weeks).

These data suggest that although intra-amniotic inflammation is causally linked to preterm labor and delivery in twin pregnancies, other mechanisms of preterm labor may also play an important role in the excessive rate of preterm labor and delivery in twin gestations.

In the current study, the adverse neonatal outcome (defined as neonatal death and/or any significant morbidity) occurred more frequently in the neonates born to mother with intra-amniotic inflammation without MIAC than those born to mother without intra-amniotic inflammation or MIAC. However, when adjusted for gestational age at delivery, there were no differences in the neonatal outcome among groups. It suggests that the difference in the neonatal outcomes among groups resulted not from intra-amniotic inflammation itself, but from the interval to delivery which was shorter in the women with intra-amniotic inflammation and/or MIAC

Ascending route of infection/inflammation in twin pregnancies

In the present study, ureaplasmas were the most common microorganisms isolated from AF, which is in keeping with the results of other studies in singleton pregnancy with preterm labor,[98-100] preterm premature rupture of membranes,[101-106] and cervical insufficiency.[50,107] Microorganisms were found in both sacs in 6 cases, and in the presenting sac alone in 3 cases. Moreover, we observed that intra-amniotic inflammation involves the presenting sac more frequently than the non-presenting sac, and that the inflammatory response was more intense in the presenting sac than in the non-presenting sac, which is in keeping with the results of other investigators[21,22]; specifically, MIAC is more frequent in the presenting sac than in the non-presenting sac in twin pregnancies with preterm labor and intact

membranes. These findings support the view that an ascending route is the common pathway of intra-amniotic infection/inflammation.

However, both sacs were involved in about two-thirds of women with intra-amniotic inflammation/MIAC. Microorganisms are frequently found in the AF of women with cervical insufficiency[25,50,108-111] and that microorganisms easily penetrate the chorioamniotic membranes *in vitro*.[112] These findings suggest chorioamniotic membranes may not be efficient barriers to prevent invasion of microorganisms.

Conclusions

The major clinical implication of our study is that the frequency of intra-amniotic inflammation in twin pregnancies with preterm labor and intact membranes is comparable to that of intra-amniotic inflammation in singleton gestations with preterm labor and intact membranes. Moreover, the pregnancy outcomes of women with intra-amniotic inflammation were as poor as those of women with proven AF infections. The presence of intra-amniotic inflammation can be detected readily whereas AF culture results may take days to become available. Therefore, modern microbiologic techniques are required to detect the presence of microorganisms more rapidly than that available through culture. We conclude that the presence of intra-amniotic inflammation appears to be a promising marker for impending preterm delivery and adverse perinatal outcome in twin pregnancies with preterm labor and intact membranes.

Table 1. Clinical characteristics of study population

Characteristics	Intra-amniotic inflammation (-)/ MIAC (-)[a] (Group 1, n=55)	P[b]	Intra-amniotic inflammation (+)/ MIAC (-)[a] (Group 2, n=26)	P[c]	Intra-amniotic inflammation (+)/ MIAC (+)[a] (Group 3; n=9)	P[d]
Maternal age, years[e]	31 (29-33)	<.001	34 (32-35)	NS	32 (29-36)	NS
Nulliparity	43/55 (78%)	NS	9/26 (35%)	NS	4/9 (44%)	.048
Gestational age at amniocentesis, weeks	30.6 (28.6-32.2)	NS	27.5 (25.1-33.3)	NS	27.7 (23.9-33.0)	NS
Cervical dilation, cm[e]	1.5 (0-3)	<.001	3.0 (1.5-4)	NS	3.0 (1.5-5)	.039
Tocolytics use[f]	50/53 (94%)	NS	21/24 (88%)	.068	5/9 (56%)	.006
Corticosteroids use[h]	43/52 (83%)	NS	14/18 (78%)	NS	5/8 (63%)	NS
Clinical chorioamnionitis	0/55	NS	0/26	NS	1/9 (11%)	NS

Data presented as median (interquartile range) or n/N (%)
MIAC, microbial invasion of amniotic cavity; NS, not significant.
[a] Intra-amniotic inflammation was defined as an elevated amniotic fluid matrix metalloproteinase-8 concentration (> 23 ng/mL) of at least 1 amniotic cavity, and MIAC was defined as a positive amniotic fluid culture of at least 1 amniotic cavity.
[b] Comparison between groups 1 and 2.
[c] Comparison between groups 2 and 3.
[d] Comparison between groups 1 and 3.
[e] P<.05 by Kruskal-Wallis ANOVA test.
[f] Four women who were unavailable for information about use of tocolytics were excluded from the analysis
[g] Administration of antenatal corticosteroid was considered when gestational age were between 23^{+0} and 33^{+6} weeks

Table 2. Hazard ratios of various variables related to amniocentesis to delivery interval

	Unadjusted hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)[a]
Intra-amniotic inflammation and/or MIAC[b]		
Intra-amniotic inflammation (-)/ MIAC (-)[b] (group 1)	Reference	Reference
Intra-amniotic inflammation (+)/ MIAC (-)[b] (group 2)	2.78 (1.67-4.62)	4.14 (2.36-7.26)
Intra-amniotic inflammation (+)/ MIAC (+)[b] (group 3)	2.69 (1.25-5.80)	4.48 (1.98-10.2)
Cervical dilatation	1.61 (0.97-2.69)	1.71 (1.01-2.89)
Gestational age at amniocentesis	1.09 (1.01-1.18)	1.17 (1.08-1.26)

CI, confidence interval; MIAC, microbial invasion of amniotic cavity.
[a] Adjusted for gestational age at amniocentesis, intra-amniotic inflammation, MIAC, and cervical dilatation
[b] Intra-amniotic inflammation was defined as an elevated amniotic fluid matrix metalloproteinase-8 concentration (> 23 ng/mL) of at least 1 amniotic cavity, and MIAC was defined as a positive amniotic fluid culture of at least 1 amniotic cavity.

Table 3. Pregnancy outcomes of study population

Characteristics	Intra-amniotic inflammation (-)/ MIAC (-)[a] (Group 1, n=55)	P[b]	Adjusted P[b,e]	Intra-amniotic inflammation (+)/ MIAC (-)[a] (Group 2, n=26)	P[c,f]	Intra-amniotic inflammation (+)/ MIAC (+)[a] (Group 3; n=9)	P[d]	Adjusted P[d,e]
Gestational age at delivery, weeks[g,h]	34.0 (31.4-35.1)	.004	-	30.2 (26.1-34.6)	NS	28.4 (25.9-33.1)	.003	-
Spontaneous delivery								
<24 hours	6/54 (11%)	.001	.002	12/26 (46%)	NS	5/9 (56%)	.006	.003
<48 hours	7/54 (13%)	<.001	<.001	16/26 (62%)	NS	5/9 (56%)	.009	.007
<7 days	17/53 (32%)	<.001	<.001	23/26 (89%)	NS	7/9 (78%)	.021	.017
Spontaneous preterm delivery								
$<34^{+0}$ weeks[i]	23/50 (46%)	.003	.021	18/21 (86%)	NS	7/8 (88%)	.053	.058
$<36^{+0}$ weeks	40/49 (81%)	NS	NS	25/26 (96%)	NS	8/8 (100%)	NS	NS
Acute histologic chorioamnionitis	12/46 (26%)	.052	.037	11/21 (52%)	NS	5/8 (63%)	.092	.084
Funisitis	2/47 (4%)	.068	.052	4/21 (19%)	NS	4/8 (50%)	.003	.003

Data presented as median (interquartile range) or n/N (%)

MIAC, microbial invasion of amniotic cavity; NS, not significant.

[a] Intra-amniotic inflammation was defined as an elevated amniotic fluid matrix metalloproteinase-8 concentration (> 23 ng/mL) of at least 1 amniotic cavity, and MIAC was defined as a positive amniotic fluid culture of at least 1 amniotic cavity.

[b] Comparison between groups 1 and 2.

[c] Comparison between groups 2 and 3.

[d] Comparison between groups 1 and 3.

[e] Adjusted for gestational age at amniocentesis and the cervical dilation (logistic regression analysis).

[f] The probability was not significant before and after adjustment for gestational age at amniocentesis and the cervical dilation.

[g] Two women whose gestational age at delivery were not known were excluded.

[h] P<.05 by Kruskal-Wallis ANOVA test.

[i] Cases with gestational age at amniocentesis $\geq 34^{+0}$ weeks were excluded from the analysis.

Table 4. Neonatal outcomes of study population

Characteristics	Intra-amniotic inflammation (-)/ MIAC (-)[a] (Group 1, n=102)	P[b]	Adjusted P[b,e]	Intra-amniotic inflammation (+)/ MIAC (-)[a] (Group 2, n=48)	P[c,f]	Intra-amniotic inflammation (+)/ MIAC (+)[a] (Group 3; n=18)	P[d]	Adjusted P[d,e]
Neonatal death	5/102 (5%)	NS	NS	6/48 (13%)	NS	4/18 (22%)	.076	NS
Significant morbidity[g,h]	19/101 (19%)	.006	.012	21/42 (50%)	NS	8/15 (53%)	.031	.086
Respiratory distress syndrome	13/101 (13%)	NS	NS	5/42 (12%)	NS	3/15 (20%)	NS	NS
Bronchopulmonary dysplasia	10/97 (10%)	.002	.015	18/42 (43%)	NS	4/15 (27%)	NS	NS
Intraventricular hemorrhage	8/101 (8%)	NS	NS	2/42 (5%)	NS	4/15 (27%)	NS	NS
Necrotizing enterocolitis	3/101 (3%)	NS	NS	4/42 (10%)	NS	0/15	NS	NS
Proven early neonatal sepsis	1/101 (1%)	NS	NS	1/42 (2%)	NS	0/15	NS	NS
Neonatal death and/or any significant morbidity	21/102 (21%)	.002	.007	27/48 (56%)	NS	11/18 (61%)	.011	.066

Data presented as n/N (%).
MIAC, microbial invasion of amniotic cavity; NS, not significant.
[a] Intra-amniotic inflammation was defined as an elevated amniotic fluid matrix metalloproteinase-8 concentration (> 23 ng/mL) of at least 1 amniotic cavity, and MIAC was defined as a positive amniotic fluid culture of at least 1 amniotic cavity.
[b] Comparison between groups 1 and 2.
[c] Comparison between groups 2 and 3.
[d] Comparison between groups 1 and 3.
[e] Adjusted for gestational age at amniocentesis and the cervical dilation (generalized estimation equation models).
[f] The probability was not significant before and after adjustment for gestational age at amniocentesis and the cervical dilation.
[g] Defined as the presence of any of the following conditions: proven early neonatal sepsis, respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage, and necrotizing enterocolitis.
[h] Ten cases were excluded from the analysis because they died shortly after delivery as a result of extreme preamaturity and thus could not be evaluated with respect to the presence or absence of neonatal morbidity.

Table 5. Relationship among various variables with adverse neonatal outcome (neonatal death and/or any significant morbidity[a])

	Unadjusted odds ratio (95% CI)	Adjusted odds ratio[b] (95% CI)	Adjusted odds ratio[c] (95% CI)
Intra-amniotic inflammation and/or MIAC[d]			
Intra-amniotic inflammation (-)/ MIAC (-)[d] (group 1)	Reference	Reference	Reference
Intra-amniotic inflammation (+)/ MIAC (-)[d] (group 2)	4.96 (1.84-13.4)	4.67 (1.59-13.7)	1.41 (0.30-6.64)
Intra-amniotic inflammation (+)/ MIAC (+)[d] (group 3)	6.06 (1.50-24.5)	5.46 (0.76-39.4)	1.09 (0.15-7.68)
Cervical dilatation	1.95 (0.68-5.56)	2.08 (0.56-7.70)	0.44 (0.09-2.24)
Gestational age at amniocentesis	0.74 (0.64-0.86)	0.74 (0.63-0.88)	1.53 (0.97-2.41)
Gestational age at delivery	0.51 (0.41-0.63)		0.33 (0.19-0.59)

MIAC, microbial invasion of amniotic cavity.

[a] Significant morbidity was defined as any of the following conditions: proven early neonatal sepsis, respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage, and necrotizing enterocolitis.

[b] Generalized estimation equation models adjusted for gestational age at amniocentesis, intra-amniotic infection, intra-amniotic inflammation, and cervical dilatation

[c] Generalized estimation equation models adjusted for gestational age at amniocentesis, intra-amniotic infection, intra-amniotic inflammation, cervical dilatation, and gestational age at delivery

[d] Intra-amniotic inflammation was defined as an elevated amniotic fluid matrix metalloproteinase-8 concentration (> 23 ng/mL) of at least 1 amniotic cavity, and MIAC was defined as a positive amniotic fluid culture of at least 1 amniotic cavity.

Table 6. Pregnancy and neonatal outcomes according to the number of amniotic cavity with an elevated matrix metalloproteinase-8 concentration

	Number of amniotic cavity with an elevated MMP-8 in a twin pair								
Characteristics	No amniotic cavity (n=55)	P[a]	Adjusted P[a,d]	One amniotic cavity (n=13)	P[b]	Adjusted P[b,d]	Both amniotic cavities (n=22)	P[c]	Adjusted P[c,d]
Spontaneous delivery									
<48 hours	7/54 (13%)	NS	NS	4/13 (31%)	.012	.015	17/22 (77%)	<.001	<.001
<7 days	17/53 (32%)	.001	.004	11/13 (85%)	NS	NS	19/22 (86%)	<.001	<.001
Spontaneous preterm delivery									
$<34^{+0}$ weeks[e]	23/50 (46%)	NS	NS	9/12 (75%)	NS	NS	16/17 (94%)	.001	.009
$<36^{+0}$ weeks	40/49 (82%)	NS	NS	12/13 (92%)	NS	NS	21/21 (100%)	.049	-
Neonatal death and/or any significant morbidity[f,g]	21/102 (21%)	.039	NS	12/24 (50%)	NS	NS	26/42 (62%)	<.001	.001

Data presented as n/N (%).

MMP-8, matrix metalloproteinase-8; elevated MMP-8, MMP-8 >23ng/mL; NS, not significant.

[a] Comparison between group without an elevated MMP-8 concentration and group with an elevated MMP-8 concentration of 1 amniotic cavity.

[b] Comparison between group with an elevated MMP-8 concentration of 1 amniotic cavity and group with an elevated MMP-8 concentration of both amniotic cavities.

[c] Comparison between group without an elevated MMP-8 concentration and group with an elevated MMP-8 concentration of both amniotic cavities.

[d] Adjusted for gestational age at amniocentesis and the cervical dilation (logistic regression analysis or generalized estimation equation analysis).

[e] Cases with gestational age at amniocentesis $\geq 34^{+0}$ weeks were excluded from the analysis.

[f] Among the study population, six twin pairs with major congenital anomaly (n=4) and unavailable data (n=2) were excluded from the analysis.

[g] Significant morbidity was defined as the presence of any of the following conditions: proven early neonatal sepsis, respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage and necrotizing enterocolitis

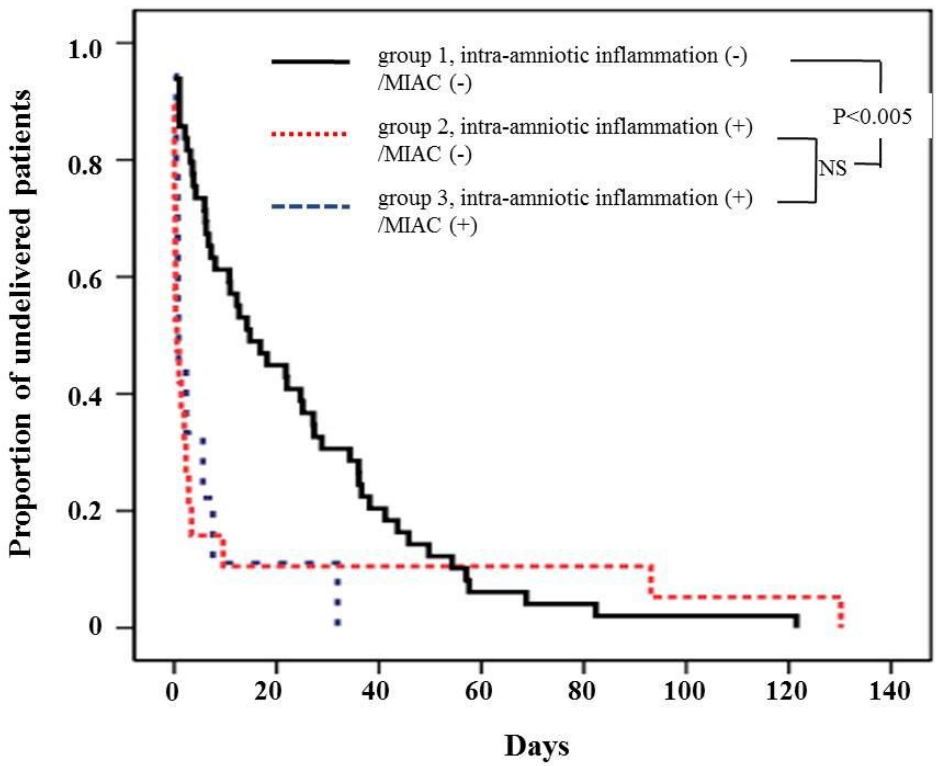

Figure 1. Survival analysis of the amniocentesis-to-delivery interval, according to the results of amniotic fluid (AF) culture and matrix metalloproteinase (MMP)-8 concentrations (group 1: median, 14 days [interquartile range, 4-36 days]; group 2: median, 1 day [interquartile range, 0-3 days]; group 3: median 1 day [interquartile range, 1-6 days]). Intra-amniotic inflammation was defined as an elevated amniotic fluid matrix metalloproteinase-8 concentration (> 23 ng/mL) of at least 1 amniotic cavity, and MIAC was defined as a positive amniotic fluid culture of at least 1 amniotic cavity.

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국문초록

조기진통을 동반한 쌍태임신에서 양수내 염증의 빈도 및 주산기 예후와의 관련성

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목적

쌍태임신은 단태임신에 비하여 주산기 사망률이 6배나 높으며, 조산이 주산기 사망과 이환의 가장 중요한 원인이다. 조기진통을 동반한 단태임신에서 사망 및 이환의 가장 중요한 원인이다. 조기진통을 동반한 단태임신에서 양수내 염증의 빈도는 약 30% 이며, 양수내 염증이 있는 경우 감염여부와 무관하게 조산으로의 진행 및 불량한 임신 예후와 관련이 있다. 이 연구의 목적은 조기진통을 동반한 쌍태임신에서 양수내 염증의 빈도 및 주산기 예후와의 관련성을 살펴보고자 함이다.

연구방법

임신 20^{+0} – 34^{+6} 주 사이의 조기진통을 동반한 90명의 쌍태임신 산모에서 양수를 천자하였다. 양수는 호기성/혐기성 세균 및 생식기 마이코플라스마 균에 대한 배양검사를 시행하였고, matrix metalloproteinase-8 (MMP-8) 농도를 측정하였다. 양수내 염증은 MMP-8의 농도가 23 ng/mL 인 경우로 정의하였다. 환자군은 양수의 분석 결과에 따라 3군으로 나누었다: 제 1군, 양수배양검사 음성이고 양수내 염증도 없는 군 (n=55); 제 2군, 양수배양검사는 음성이나 적어도 한 양막강에서 양수내 염증이 있는 경우 (n=26); 제 3군, 적어도 한 양막강에서 양수 배양검사 양성이고 양수내 염증이 있는 경우 (n=9).

결과

1) 조기진통을 동반한 쌍태임신에서 양수내 염증 (한 양막강 이상)의 빈도는 39% (35/90) 이었다. 양수내 감염 (한 양막강 이상)의 빈도는 10% (9/90)이었다. 양수내 감염이 없이 염증만 발견된 경우도 29% 였다 (26/90); 2) 두 양막강 모두에서 양수내 염증이 있는 경우가 22예, 선진부 양막강에만 양수내 염증이 있는 경우가 12예, 비-선진부 양막강에만 양수내 염증이 있는 경우가 1예였다; 3) 양수내 감염이 발견 된 9예 중, 두 양막강 모두 감염이 있는 경우가 6예, 비-선진부 양막강에만 감염이 있는 경우가 3예였다; 4) 감염이 없더라도 양수내 염증이 있는 산모군은 감염 및 염증이 모두 없는 산모군에 비하여, 불량한 예후 (이른 분만 주수, 짧은 양수천자-분만 간격, 신생아 사망 또는 이환)의 빈도가

유의하게 증가하였다. 감염은 없으나 양수내 염증이 있는 군과 양수내 감염이 있는 군간의 유의한 예후 차이는 없었다.

결론

1) 조기진통을 동반한 쌍태임신에서 양수내 염증의 빈도는 39% 였으며, 조산 및 불량한 주산기 예후의 위험인자이다. 2) 양수내 염증은 선진부 양막강에서 비-선진부 양막강에 비하여 보다 흔히 발견되며, 이는 상행성 경로가 양수내 염증의 일반적 경로임을 뒷받침 하는 소견이다.

주요어: 양수내 염증, 양수내 감염, 조기진통, 조산, 쌍태임신